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Distribution and prevalence of *Wolbachia* in introduced populations of the fire ant *Solenopsis invicta*

A. M. Bouwma*†, M. E. Ahrens†, C. J. DeHeer‡ and D. DeWayne Shoemaker†

†*Department of Entomology, University Wisconsin-Madison, Madison, USA;* ‡*Department of Entomology, North Carolina State University, Raleigh, USA*

Abstract

Wolbachia **are intracellular bacteria that induce phenotypic effects in many arthropod hosts to enhance their own transmission within host populations.** ***Wolbachia*** **commonly infect the Red Imported Fire Ant,** ***Solenopsis invicta*****, in native South American populations. A previous study failed to detect** ***Wolbachia*** **in fire ants from the introduced range in the USA. We conducted an extensive study of individuals collected from 1157 nests from 10 widespread geographical populations in the USA.** ***Wolbachia*** **were detected in ants from two nearby populations in southern Mississippi, with different variants (*wsp* gene sequences) infecting ants from colonies of the multiple-queen (polygyne) vs. single-queen (monogyne) social forms. The parsimonious explanation for the presence of** ***Wolbachia*** **in introduced** ***S. invicta*** **is that there have been one or more recent introductions of** ***Wolbachia*****-infected fire ants into the southern USA.**

Keywords: Fire ants, ***Solenopsis invicta, Wolbachia*****.**

Introduction

Wolbachia are intracellular bacteria found in a wide range of arthropods and filarial nematodes (Bandi *et al*., 1998; O'Neill *et al*., 1992). These bacteria are primarily maternally transmitted, and many variants enhance their transmission within host populations by a variety of mechanisms, termed phenotypic effects, that either distort the sex ratio of their host's offspring in favour of females (parthenogenesis, feminization of males, male-killing), or decrease the reproductive success of uninfected female hosts (cytoplasmic incompatibility) (Werren, 1997). The strong manipulation of host reproduction by *Wolbachia* enables this microbe to spread even if it induces a physiological cost that reduces fitness in its hosts (Turelli, 1994).

Wolbachia infections are common in ants (Van Borm *et al*., 2001; Wenseleers *et al*., 1998; Jeyaprakash & Hoy, 2000; Shoemaker *et al*., 2000). Further, *Wolbachia* variants found in New World ants are strikingly similar to each other (based on the highly variable *Wolbachia* outer surface protein gene *wsp*) yet different from all currently known *Wolbachia* variants in other insect groups suggesting that these *Wolbachia* variants are ant specialists (Tsutsui *et al*., 2003).

Three New World *Wolbachia* host ants, the Argentine ant *Linepithema humile* (Reuter *et al*., 2005; Tsutsui *et al*., 2003), and the Red and Black Imported Fire Ants, *Solenopsis invicta* and *S. richteri* (Shoemaker *et al*., 2003, 2000), have been introduced independently into North America where they have become significant economic pests. Recent studies by Tsutsui *et al*. (2003) and Reuter *et al*. (2005) suggest that *Wolbachia* infections may have been lost during the colonisation of new habitats by Argentine ants since *Wolbachia* infections are common in the native range of this species, but rare or absent among introduced individuals. Similarly, Shoemaker *et al*. (2003, 2000) found that *Wolbachia* infections are common in numerous native populations of the two fire ant species *S. invicta* and *S. richteri*, but apparently absent in introduced populations in the USA (however, see Jeyaprakash & Hoy, 2000). While the phenotypic and fitness effects induced by the *Wolbachia* in these three ant species are as yet unknown, these studies raise the possibility that the absence of this microbe contributes to the success of these ants in their introduced range (Shoemaker *et al*., 2000; Tsutsui *et al*., 2003).

While *Wolbachia* infections are common in *native S. invicta*, *Wolbachia* prevalence varies considerably among different geographical populations (Shoemaker *et al*., 2003), suggesting that similar geographical variation in *Wolbachia* prevalence occurs among introduced populations. If so, the previously reported absence of *Wolbachia* infections among *S. invicta* in the USA may be due to inadequate geographical sampling. Indeed, a recent study by Jeyaprakash & Hoy (2000), using a highly sensitive long PCR assay, found *Wolbachia* in a single *S. invicta* individual sampled

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*Correspondence: Andrew M. Bouwma, Department of Entomology, University of Wisconsin-Madison, 643 Russell labs, 1630 Linden Drive, Madison, WI 53706, USA. Tel.: +1 608 265 6128; fax: +1 608 262 3322; e-mail: bouwma@entomology.wisc.edu

from a laboratory colony (presumably from Florida, USA). For the present study we conducted an extensive survey for *Wolbachia* in introduced *S. invicta*, using both the long PCR protocol of Jeyaprakash & Hoy (2000) and a standard PCR assay (Shoemaker *et al*., 2003; Zhou *et al*., 1998). In our study, the different PCR assays produced identical results, and while individuals from most populations were uninfected, multiple individuals from two populations in Mississippi, USA did harbour *Wolbachia* infections. Surprisingly, sequence analyses revealed that two *Wolbachia* variants occur in the USA, both of which are identical to variants previously described in native *S. invicta* (Shoemaker *et al*., 2000), yet differ from the variant found in *S. invicta* by Jeyaprakash & Hoy (2000).

Results

We did not detect *Wolbachia* in any individuals from eight of the 10 *S. invicta* populations studied. However, 11 individuals from two nearby populations in southern Mississippi (Hurley and Pascagoula) tested positive for *Wolbachia* (Fig. 1, Table 1). In samples collected in 1996, ants from eight of 61 colonies (13%) of the polygyne social form from Hurley, Mississippi harboured *Wolbachia*, whereas individuals from all 59 monogyne colonies from this same site were uninfected. (Monogyne colonies possess a single egg-laying queen, whereas polygyne colonies possess several to hundreds [Glancey *et al*., 1973]; there is limited gene flow between these two social forms [Ross & Shoemaker, 1993, 1997; Shoemaker & Ross, 1996]). In samples collected from Hurley, southern Mississippi in 2003, we did not find any infected ants from 76 polygyne colonies, however *Wolbachia* were detected in one individual from one of the 82 monogyne colonies (1.2%) at this site. In the same year, *Wolbachia*-infected individuals from two of 41 monogyne colonies (4.9%) in Pascagoula, Mississippi were observed, while a single individual from a polygyne colony was uninfected (Fig. 1, Table 1).

Sequence analyses of the *Wolbachia* variants (*wsp*; *Wolbachia* surface protein) from the 11 infected individuals (colonies) yielded one major result: all infected individuals

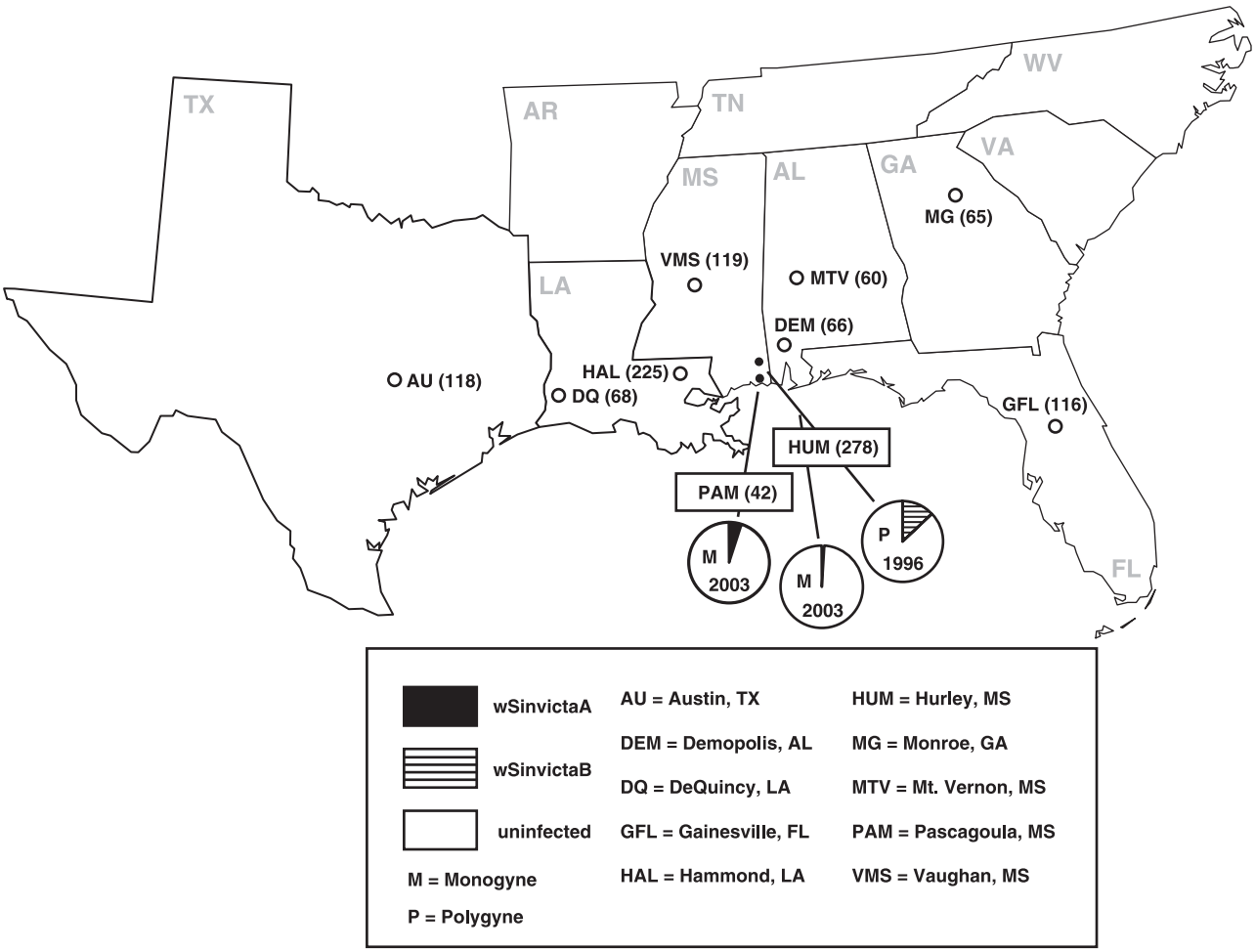

Figure 1. *Wolbachia* infection prevalence in introduced populations of *Solenopsis invicta* in the USA. All populations are uninfected unless indicated otherwise.

Table 1. Prevalence of *Wolbachia* infections in introduced populations of the fire ant *Solenopsis invicta* in the USA

Location	Social form M = Monogyne P = Polygyne	Year	*N*	Prevalence of *Wolbachia* infections	95% CI
Austin, TX	M	1996	59	0	0, 0.05
	P	1996	59	0	0, 0.05
DeQuincy, LA	M	1996	43	0	0, 0.07
	P	1996	25	0	0, 0.12
Hammond, LA	M	1996	64	0	0, 0.05
	P	1996	55	0	0, 0.05
	M	2003	62	0	0, 0.05
	P	2003	44	0	0, 0.07
Hurley, MS	M	1996	59	0	0, 0.05
	P	1996	61	0.13	0.05, 0.22
	M	2003	82	0.01	0, 0.04
	P	2003	76	0	0, 0.04
Pascagoula, MS	M	2003	41	0.05	0.02, 0.08
	P	2003	1	0	
Vaughan, MS	M	2003	64	0	0, 0.05
	P	2003	55	0	0, 0.05
Demopolis, AL	M	2003	3	0	
	P	2003	63	0	0, 0.05
Mt. Vernon, AL	M	2003	59	0	0, 0.05
	P	2003	1	0	
Monroe, GA	M	1995	34	0	0, 0.09
	P	1995	31	0	0, 0.09
Gainesville, FL	M	1996	60	0	0, 0.05
	P	1996	56	0	0, 0.05

from polygyne colonies (1996 Hurley, [*n* = 8]) had an identical *Wolbachia* variant, as did all of the *Wolbachia*-infected individuals from monogyne nests (2003 Hurley, [*n* = 1] and 2003 Pascagoula, [*n* = 2]). The variants infecting individuals of each social form, however, were quite different from each other. Specifically, the *Wolbachia* variant in infected ants from monogyne colonies was identical to a variant known to infect native *S. invicta* (wSinvictaA [AF243435]) and belongs to the *InvA* subgroup of fire ant *Wolbachia* (*Wolbachia* supergroup A) whereas the variant in ants from polygyne colonies is identical to another variant known to infect native *S. invicta* (wSinvictaB [AF243436]) but belongs to the divergent *InvB* subgroup (*Wolbachia* supergroup B) (Fig. 1).

Discussion

Our study revealed that two of 10 sampled populations of *S. invicta* in the USA harbour one of two *Wolbachia* variants at low prevalence (1–13%). The only previous evidence for *Wolbachia* in introduced *S. invicta* comes from a study by Jeyaprakash & Hoy (2000) in which the authors reported a single *Wolbachia*-infected individual from a laboratory colony presumably collected in Gainesville, Florida, USA. These authors demonstrated that a long PCR protocol was more sensitive at detecting *Wolbachia* in a diversity of hosts. We used this same protocol in our study of *Wolbachia* infections in 116 colonies in Gainesville, Florida USA (as well as a subset of colonies from other locations) in order to see if any infections escaped detection simply due to lack of sensitivity of our standard PCR assay. However, the long PCR assay produced identical results to our standard assay in all cases, and both assays failed to detect *Wolbachia* within individuals from any of these colonies. Thus, the *Wolbachia* variant found by Jeyaprakash & Hoy (2000) presumably was rare enough to escape detection or has recently been lost from natural *S. invicta* populations in Gainesville, Florida.

The low prevalence of *Wolbachia* infections is of particular interest since some theoretical models predict that such low frequencies likely are unstable (Turelli, 1994), suggesting that these *Wolbachia* infections are either in the process of sweeping through fire ant populations in the southern USA or are on the verge of being lost. (While male-killing *Wolbachia* variants may persist at a low equilibrium prevalence, the *Wolbachia* variants in fire ants likely do not induce this phenotype since males are commonly infected [Shoemaker *et al*., 2000]). Indeed, for one of the sites where we have data for two collections from different years (i.e. the polygyne population from Hurley, Mississippi), *Wolbachia* infection prevalence was significantly lower in 2003 than in 1996 (non-overlap of 95% confidence intervals). Nonetheless, we cannot rule out that the observed difference in *Wolbachia* prevalence is due to microgeographical variation rather than temporal variation in prevalence since the collection locations in Hurley, Mississippi were not the same in 1996 and 2003 (samples came from neighbouring pastures), and polygyne *S. invicta* consistently exhibit high levels of mtDNA genetic structure even at small spatial scales (Ross & Shoemaker, 1997).

We see three possible scenarios that could explain the restricted distribution of *Wolbachia* in *S. invicta* to individuals from Pascagoula and Hurley, Mississippi: (1) the *Wolbachia* variants we found in Hurley and Pascagoula were present in some proportion of the original monogyne founders introduced into the USA in the late 1930s or polygyne founders in the early 1970s that subsequently became established, but the infection has failed to spread throughout the USA; (2) a more recent introduction of *Wolbachia*-infected queens of *S. invicta* into the southern USA has occurred subsequent to the original invasion of ants representing each social form; or (3) *Wolbachia*-infections have been recently horizontally transmitted into introduced *S. invicta* from another host in the USA.

We consider the second scenario the most likely explanation for the distribution of *Wolbachia* infections in introduced *S. invicta* for several reasons. Firstly, if a proportion of the original monogyne foundresses introduced into Mobile, Alabam in the 1930s, or polygyne foundresses in the 1970s, were infected with *Wolbachia*, one might predict that *Wolbachia* infections would have spread throughout the USA along with the rapid spread of the ants shortly after their initial invasion. However, we failed to detect *Wolbachia*

infections in ants from a total of 837 nests collected outside of two nearby populations in Mississippi. In addition, *Wolbachia* infections were present in individuals from locations that are within 65 km of a likely port of entry (Mobile, Alabam), consistent with a recent introduction. The finding of two very different *Wolbachia* variants that are each associated with different social forms in the USA suggests that there likely have been two separate, recent introductions of *Wolbachia*-infected ants involving each of the two social forms. However, because both of these *Wolbachia* variants are found in sympatric native populations of each social form of *S. invicta* (Ahrens & Shoemaker, 2005), we cannot rule out completely the possibility of a single introduction involving queens of both social forms and both *Wolbachia* variants.

We consider the third scenario of horizontal transmission from another host the least likely. While the *Wolbachia* variant found by Jeyaprakash & Hoy (2000) has never been found in another ant and probably came from a non-ant host (Tsutsui *et al.*, 2003), the two different *Wolbachia* variants we found in introduced *S. invicta* are known to infect *S. invicta* from South America (Shoemaker *et al.*, 2003). Further, these two variants are associated with the same mtDNA haplotypes in the USA as they are in South America (D. Shoemaker, unpublished data), and have never been found in another insect. Thus, the parsimonious explanation is that these *Wolbachia* variants in introduced *S. invicta* are the result of one or more recent introductions of *Wolbachia*-infected *S. invicta* into the USA.

The two infected USA populations of *S. invicta* may serve as model systems for studying the dynamics and phenotypic effects of *Wolbachia* infections in naturally infected host populations, an opportunity rarely afforded to researchers (Hoshizaki & Shimada, 1995; Turelli & Hoffmann, 1991). Our study is an important first step toward collecting the necessary baseline geographical and temporal data on *Wolbachia* prevalence in introduced populations of fire ants that will allow us to determine both the fate of these infections and their impact on populations in the introduced range of *S. invicta*.

Experimental procedures

Collection of ants

Over three separate collecting trips in the spring of 1995, 1996 and 2003, we collected ants representing different life stages and castes from 1157 colonies at a total of 10 locations distributed throughout the range of introduced *S. invicta* in the southern USA (Fig. 1, Table 1). For each site, we included only nests located within 40 km of all other nests sampled at that site. We placed all collected ants immediately into liquid nitrogen in the field and subsequently stored them at −80 °C pending DNA analyses. We also made an effort to collect colonies representing both social forms of *S. invicta* from each site. We approximated social form in the field using cues from nest density, worker size, and nest brood composition (Ross & Shoemaker, 1997), and then subsequently confirmed it in the laboratory using one of two methods described below.

Screening for Wolbachia

We isolated total genomic DNA from individual ants and bulk samples (see below) using the Puregene DNA isolation kit (Shoemaker *et al.*, 2000) (Gentra Systems Minneapolis, Minnesota). Shoemaker *et al.* (2003) showed that *Wolbachia* infections in *S. invicta* are transmitted to the offspring with nearly 100% fidelity, which means that screening one individual is a reliable method for determining whether individuals within a monogyne colony are infected. However, because the offspring within polygyne colonies are produced by multiple, unrelated queens, this procedure measures the proportion of infected polygyne queens in the population rather than the proportion of *Wolbachia*-infected polygyne colonies. We screened total genomic DNA from each ant for the presence of *Wolbachia* using PCR with the primers *Wsp*81F and *Wsp*691R (Zhou *et al.*, 1998). Details of the PCRs, PCR profiles, and electrophoresis of products are described in Shoemaker *et al.* (2003, 2000). For all of the colonies collected from Florida ($n = 116$) and subset of the other colonies ($n = 298$), we also screened for the *wsp* gene and the presence of *Wolbachia* using the primers *Wsp*-Forward and *Wsp*-Reverse following the long PCR protocol detailed in Jeyaprakash & Hoy (2000).

Determination of social form

For all samples collected in 1995 and 1996, we confirmed polygyny by finding two or more wingless (reproductive) queens in a nest, by detecting multiple families represented among eight or more nestmate offspring females assayed at six polymorphic allozyme loci, and/or by detecting the presence of the b allele of the gene *Gp-9*, which occurs only in the polygyne social form (Krieger & Ross, 2002; Ross, 1997). We confirmed monogyny by detecting a single family represented among eight or more nestmate females assayed at the six allozyme loci and by failing to find the $Gp\text{-}9^b$ allele among these females (DeHeer & Tschinkel, 1998). For the colonies we collected in 2003, we determined social form using a 2-stage, allele-specific PCR assay at the gene *Gp-9* designed to amplify 'b'-like alleles only, (see Krieger & Ross, 2002; Ross *et al.*, 2003). For those colonies that were negative for the stage-2 PCR, we performed bulk extractions of an additional five individuals and repeated the 2-step PCR assay to confirm monogyny.

Sequencing of Wolbachia *strains*

We sequenced a portion of the *wsp* gene from all *Wolbachia*-infected individuals in order to identify the *Wolbachia* variants infecting these ants. We PCR-amplified *Wolbachia* DNA using the primers *Wsp*81F and *Wsp*691R. PCR reaction components, thermal cycling conditions, and sequencing methods were identical to those described in Ahrens & Shoemaker (2005).

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